membranes composed of DOPC/sphingomyelin/cholesterol were prepared on ordered pore-arrays in silicon with different pore diameters by spreading and fusion of giant unilamellar vesicles. To induce vesicle rupture and fusion, the top part of a gold-covered silicon substrate was functionalized with a thiol-bearing cholesterol derivative that renders the surface hydrophobic. Confocal laser scanning fluorescence microscopy was used to investigate the phase behavior of the obtained pore-spanning membranes. Coexisting liquid-ordered- and liquid-disordered domains were visualized for DOPC/sphingomyelin/cholesterol (40:40:20) membranes. The same result was obtained for lipid mixtures, in which 5 mol% of sphingomyelin was replaced by 5 mol% of the glycolipid Gb3. Videomicroscopy on these domains demonstrated their lateral mobility on the surface. The size of the lo-phase domains was strongly affected by the underlying pore size of the silicon substrate and could be controlled by temperature, and the cholesterol content in the membrane, which was modulated by the addition of methyl-β-cyclodextrin. Gb3 served as receptor for Shiga toxin B-pentamers, which bind to the membranes and thus considerably modulate the phase behavior of the pore-spanning membranes.

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Cholesterol Dependent and Independent Domain Formation in Mixed Phosphatidylinositol/Phosphoinositide Model Membranes

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Phosphoinositides (PIPs) have been shown to mediate a large variety of membrane trafficking events. Phosphatidylinositol (PI) is involved in these signaling events since it is a precursor for PIPs. In addition to this role, we hypothesize that the interaction of PI with PIPs affects the lateral distribution of PIPs and hence the spatial organization of the respective signaling event. The spatial organization of PI/PIPs is expected to be further modulated by the presence of cholesterol. To explore these questions, we studied the effect of PI on PIP domain formation in the absence/presence of cholesterol. While binary mixtures of PC/PI or PC/PIP_2 did not reveal any macroscopically discernable domains, we observed in the presence of cholesterol the formation of PI and PI(4,5)P_2 enriched domains. For cholesterol derivatives such as cholestenone or cholesterylester, we did not observe the formation of domains, indicating that the cholesterol hydroxyl group is an important factor for the observed interaction between the sterol and the lipid. We extended our studies to ternary mixtures of PC/PI/PI(4,5)P_2, which showed also in the absence of cholesterol domain formation for physiologically relevant concentrations of the anionic lipids. We believe that the presence of PI "dilutes" the high negative charge found at the PI(4,5)P_2 headgroup, allowing PI and PI(4,5)P_2 to co-localize in domains. Using fluorescence microscopy measurements of GUVs and monolayers at the air/water interface, we extended this study further and investigated the effect of cholesterol on the morphology of these ternary lipid systems.

135-Plat

How Lipids use Short, Strong H-Bonds for Membrane Stability, Making ATP & More

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Short, strong H-bonds (SSHB's) were first recognized by F. H. Westheimer and O. T. Benfey in 1956. They consist of multi-oxygen anions such as carboxyls, phosphates or sulfates forming tight H-bonds, with other multi-oxygen anions. Westheimer called them acid-anions because the resulting complex was both an acid and a basic anion. He was studying the pK shifts of maleic acid (cis-2-succinate) pK's (pK1 = 1.9, pK2 = 6.3) vs. those of fumarate (trans-2-succinate) pK1 = 3.1, pK2 = 4.6). He explained the remarkable difference by the fact that the maleate carboxyls are rammed against each other trapping a proton between the anions, using resonance of the 4 oxygens sharing a charged electron by resonance. Thus maleic acid has only one charge and 2 carboxyls between pH 2.0 and 6.8. These H-bonds vary in length from 2,45 to 2.65 Å and have been detected in proteins by 1) highly deshielded resonances (15-20 ppm); D/H fractionation factors << 1.0; solvent exchange rates <<1 order of magnitude; & H-bond strength >5kcal/mol. Typical H-bonds in proteins and nucleic acids are 2.7-3.0Å in length and 2 ± 1 kcal/mol in strength. SSHBs are commonly held proximal by external forces such as protein conformation or hydrophobic forces between chains. They are not typically observed by X-ray measurements, however the protons are far up-field in proton NMR. Examples will be discussed on the stability fatty acid vesicles, the use of cardiolipin's conformation in bilayers, the stability of chlorosulfolipid membranes of Chrysomonads and in the stability of other example membranes.

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Lipid Raft Formation and Properties are Necessary and Sufficient to Explain the Properties of Membrane Domains in *B. Burgdorferi* and are Necessary for its Membrane Integrity

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In eukaryotic cells liquid ordered (Lo) microdomains (rafts) rich in sphingolipids and cholesterol are believed to have an important role in many membrane functions. *Borrelia burgdorferi (B.b),* the bacterium that is the causative agent of Lyme disease, contains host-derived free cholesterol and large amounts of cholesteryl glycolipids. We recently found that microdomains large enough to be seen by transmission electron microscopy (TEM) form in its outer membrane (La Rocca et al (2010) Cell Host and Microbe 8, 331-342). In this study, the extent to which *B.b.* membrane domains have lipid raft properties was investigated. We carried out lipid substitutions in *B.b.* cells in which cholesterol lipids were partly substituted with various sterols having differing abilities to support ordered domain formation. TEM was used to visualize *B.b.* membrane domains, while in live, unfixed *B.b.* cells FRET and fluorescence anisotropy were used to detect domain segregation and measure overall membrane order, respectively. Both in ordinary *B.b.* cells and in cells after substitution with sterols that promote ordered domain formation in model membranes there was domain segregation and membrane order consistent with liquid ordered/liquid disordered domain co-existence. in contrast, after substitution with sterols that do not support ordered domain formation in model membranes there was no domain segregation and membrane order was more consistent with the liquid disordered state. Association of biotinylated lipids with *B.b.* domains required that they contain saturated rather than unsaturated acyl chains. Membrane integrity and growth after sterol substitution also required raft-forming sterols. Sterols not supporting ordered domain formation resulted in osmotic lysis of the organisms. These properties demonstrate that *B. burgdorferi* membrane domains have the properties of lipid rafts.

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Sphingolipid-Enriched Microdomains in the Plasma Membrane of *Saccharomyces Cerevisiae:* Ergosterol-Free «Lipid Rafts» in the Gel Phase

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The plasma membrane (PM) of *Saccharomyces cerevisiae* was studied using the probes *trans*-parinaric acid (t-PnA) and diphenylhexatriene (DPH), providing the first direct evidence for the presence of gel domains in living cells and showing that the PM is mostly constituted by ordered domains[(1)]. The fluorescence lifetimes of t-PnA are particularly sensitive to the presence and nature of ordered domains, and here the first measurements in yeast cells are reported. A long fluorescence lifetime typical of the gel phase (> 30 ns) was found in mid-exponential phase wild-type (wt) cells from two different genetic backgrounds, at both 24 and 30°C. The confirmation of the gel nature was achieved by comparing t-PnA and DPH fluorescence anisotropy, and studying the thermotropic transitions of liposomes reconstituted from PM lipid-extracts. To address the location and composition of the domains spheroplasts, the isolated PM, liposomes from total lipid-extracts, and living cells of several mutant strains with deletions in genes coding for enzymes involved in ergosterol-, sphingolipid- and GPI-anchor biosynthesis were also studied. It was shown that the gel domains are not ergosterol-enriched lipid rafts, but mainly composed of sphingolipids and GPI-anchored proteins, suggesting important roles in membrane traffic, signaling, and interactions with the cell wall. The findings provide a biophysical mechanism for the targeting of some proteins to the PM, which is sphingolipid-dependent but sterol-independent and for the formation of membrane compartments in the yeast PM (MCP and MCC)[(2)].

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